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Breast Cancer: a Preclinical Study

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13. Abstract (Maximum 200 Words) <i>(abstract should contain no proprietary or confidential information)</i> Overexpression of Her-2/neu is one of the major alterations in breast cancer and is associated with metastatic disease, poor prognosis and overall survival. Her-2/neu is a self antigen, therefore, self-tolerance is a major burden for the induction of effective antitumor immune responses. Clinical trials show that immunizations with neu-antigens do not promote a potent protective mediated immune response. In order to develop a therapy to target Her-2/neu expressing tumors, we have devised a strategy where we can endow T cells with antitumor specificity and bypass the effect of self-tolerance. We developed an approach by genetic engineering where we have genetically altered T cell populations to express high affinity T cell receptors (TCR) chains specific for Her-2/neu. High affinity TCR were obtained from A2.1 transgenic mice immunized with A2.1-Her-2neu immunodominant epitopes. The TCR genes from the CTLs were cloned and engineered to be expressed on T cells. T cells expressing these engineered TCR can recognize and kill Her-2/neu expressing breast tumors. These results demonstrate a novel strategy to target Her-2/neu positive tumors for the treatment of breast cancer.				
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Table of Contents

Cover.....	1
SF 298.....	2
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	6
Reportable Outcomes.....	6
Conclusions.....	6
References.....	6
Appendices.....	6-7

Introduction

T cell tolerance to self is a fundamental process evolved within the immune system to prevent reactivity and damage to self tissues. Tolerance induction toward many self proteins occurs centrally during T cell development within the thymus. However, peripheral extrathymic tolerance mechanisms exist to prevent reactivity toward tissue specific antigens not normally found in the thymus nor found in the circulation. An accumulating body of evidence suggests that many potential tumor associated antigens spanning a number of different tumor types are in fact overly expressed

normal gene products. One such example relevant to this application is HER-2/neu which is overexpressed in 25-35 % of mammary carcinomas in humans. Based on results from clinical trials with neu-antigens, one important prediction is that deliberate immunization with Her-2/neu antigen formulations in patients afflicted with breast, ovarian or possibly other cancers may be incapable of promoting a potent protective cell mediated immune response. Thus, strategies designed to induce antitumor immune responses against self-tumor

antigens like Her-2/neu may not be sufficient to eradicate tumors because of self-tolerance. In light of these considerations, it would be of value to identify alternative immunotherapeutic strategies to target self tumor antigens such as Her-2/neu.

We have previously identified two immunodominant HLA-A2.1 restricted epitopes of HER-2/neu, p369 (amino acids 369-377) and p773 (amino acids 773-781) utilizing the HLA-A2.1 transgenic mice. We have prepared T cell clones against these peptides and these clones are of high affinity. Importantly the p369 or p773 specific/ HLA-A2.1 restricted CTL efficiently lysed human HLA-A2.1 HER-2/neu⁺ tumors (Fig 1).

Fig. 1

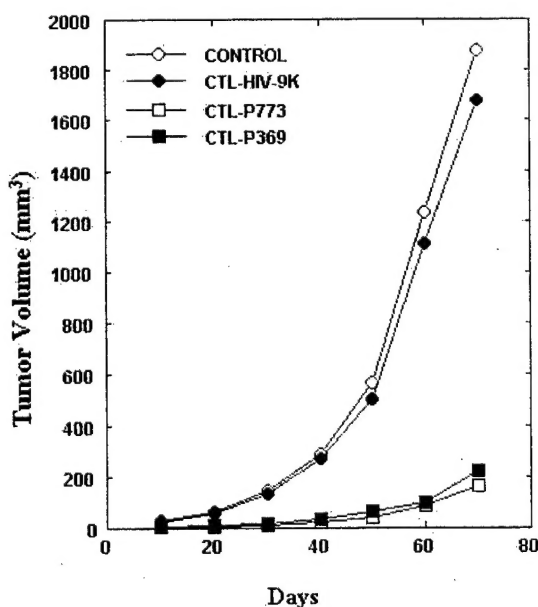
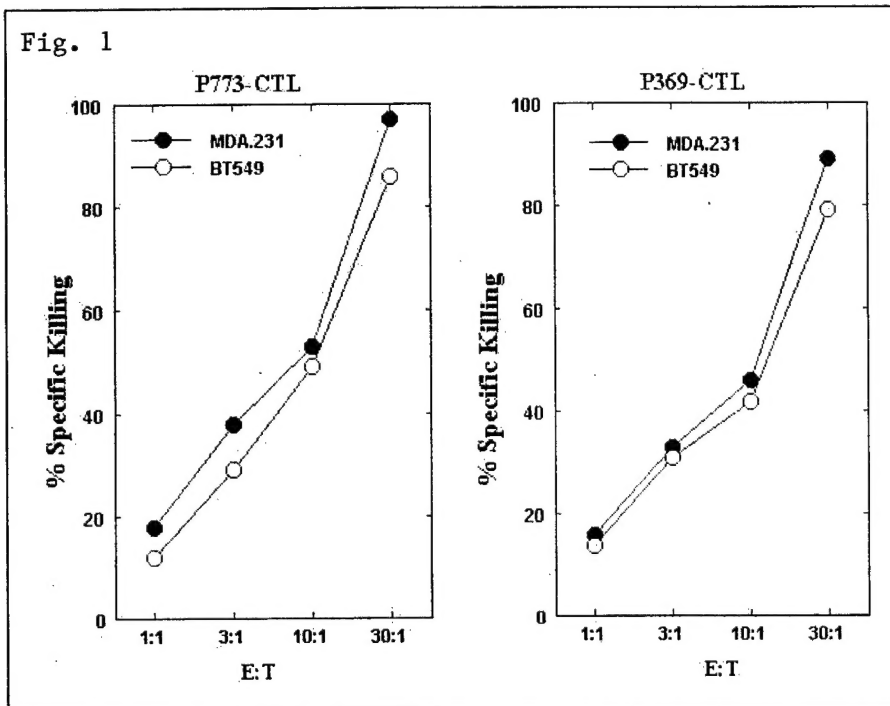


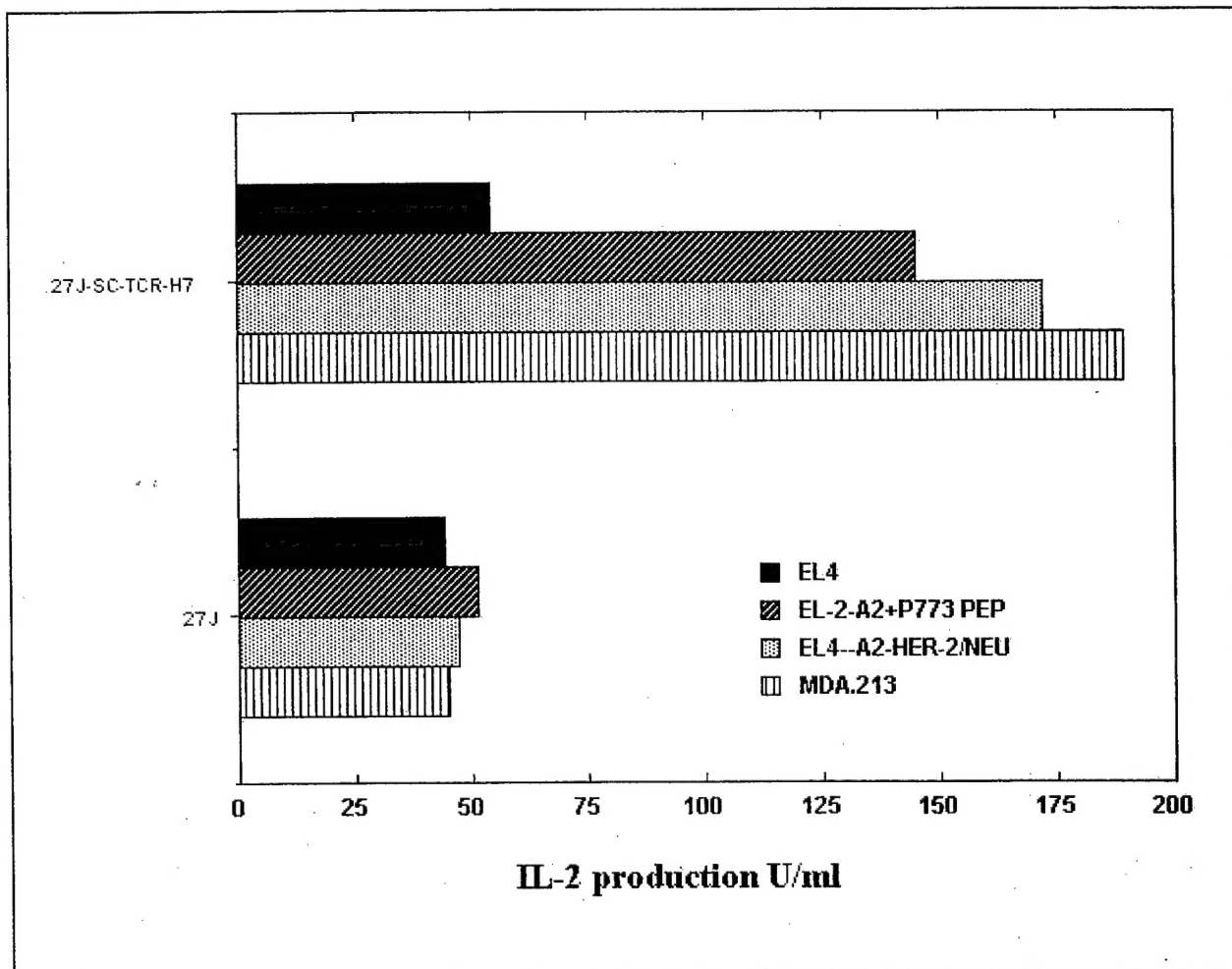
Fig. 2.

These same two clones were then tested *in vivo* for their ability to block growth of MDA-MB231, an HLA-A2.1⁺/HER-2/neu⁺ human breast carcinoma cell line. In these experiments 50 x 10⁶ T cell clones were adoptively transferred into nude mice at days 7 and 14 following subcutaneous injection of 2 x 10⁶ MDA-MB231 tumor cells. As shown in the Figure 2 above, control mice or mice receiving an irrelevant CTL line specific for an HIV-polymerase peptide developed tumors that were measurable by days 40-45. In contrast, mice receiving either the p369 CTL clone or the p773 CTL clone showed only minimal tumor growth measurable only by days 62-70.

We hypothesize that by expressing the T cell receptors (TCR) from the CTLs derived from the A2.1 transgenic mice it may be possible to render new antitumor specificity to T cells which can recognize tumors with high affinity. To this end we cloned the TCR genes from the p773 CTLs and we developed chimeric molecules to be expressed on T cells.

Body

***In vitro* and *In vivo* Killing of Human HLA-A2.1⁺ / HER-2/neu⁺ Tumors by p369 and p773 Specific CTL Clones:** Murine CD8⁺ T cell clones specific for the p369 and p773 peptides of HER-2/neu recognize are able to lyse very efficiently two representative HLA-A2.1⁺ / HER-2/neu⁺ human breast tumor cell lines (Fig 1).



Cloning and Expression of p369 and p773 Specific TCR α and β Chains:

In our initial attempts to confer specificity on T cell populations via TCR $\alpha\beta$ gene transfer, we cloned the V α and V β genes of the TCR from the p773 CTL (Fig 3). Next, we constructed a chimeric TCR molecule. This molecule consisted of a single chain TCR (scTCR) in which the V α and V β domains derived from the p773 CTL clone were joined by a flexible linker, (Gly4Ser)₃, followed by the transmembrane and cytoplasmic domains of the CD3 ζ chain. The TCR chains expressed by the p773 clones were identified by 5' RACE-PCR and were found to be encoded by V α 1 and V β 8.3. (Also using 5' RACE-PCR, we have identified and cloned the V α 10 and V β 8 variable regions genes of the p369 specific CTL clone, data not shown). To determine whether the p773 scTCR- ζ chimeric molecules were functional, we transfected a TCR^{CD4}^{CD8} T cell hybridoma, MD-45-27J, with the plasmid preparation encoding the scTCR- ζ chimera. Single cell clones that grew under G418 selection were screened for IL-2 secretion following stimulation with MDA.231 human breast cell line, EL4 cells transfected with A2 and Her-2/neu or EL4-A2 cells pulsed with the p773 peptide. Results using a representative clone are shown in Fig. 4. It can be seen that the scTCR- ζ transfectant was able to respond specifically to the peptide as well as to cells expressing both A2.1 and Her-2/neu.

Key Research Accomplishments, Reportable Outcomes and Conclusions

It is important to point out that we demonstrate with these experiments that it is possible to provide new antitumor specificity to T cells with the construction of the sc-TCR. It is important to point out that the sc-TCR transfectants were able to respond to human tumors without co-expression of CD8. These data might suggest that these TCR chains are of sufficient affinity that they can function in normal T cells without the requirement of CD8, albeit perhaps at lower efficiency. The next step is to evaluate the expression of these constructs into human T cells. It is very important to assess the antitumor effect of transduced T cells from breast cancer patients and evaluate whether these T cells can eradicate tumor in vivo. Once we evaluate this approach in the pre-clinical model we could have a better idea on the effectiveness of this approach, and the information gained from these studies could form the basis for subsequent clinical studies.

Overall, these studies allowed us to prove that we can provide new antitumor specificity to T cells and that was our goal.

Bibliography

No papers or meeting abstracts have resulted from this study.

Personnel List

The following personnel have received pay from the research effort: Daniel P. Gold, Ph.D. and Joseph Lustgarten, Ph.D.

Appendix

1 full page figure (next page)

171
 ATG AAG AGC CTG CTG AGG TCT CTG CTG GGG CTT CTG TGC ACC CAG GTT TGC TGG GTG AAA GGA CAG CAA GTG CAG CAG AGC CCC GCG TCC
 M K S L L S S L L G L L C P Q V C W V K G Q Q V Q Q S P A S
 91/31
 TTG GTT CTG CAG GAG GGG GAG AAC GCA GAG CTG CAG TAT AAC ITT TCC ACA TCT TTG AAC AGT ATG CAG TGG TTT TAC CAA CGT CCT GGG
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 271/91
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 S L D I S S S Q T T D S G T Y L C A L V E L K P G E A Y L W
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 T G N Q V I Y Q A G G G G S G G G G S G G G G S G G G G S A V T Q S P
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 1261/421
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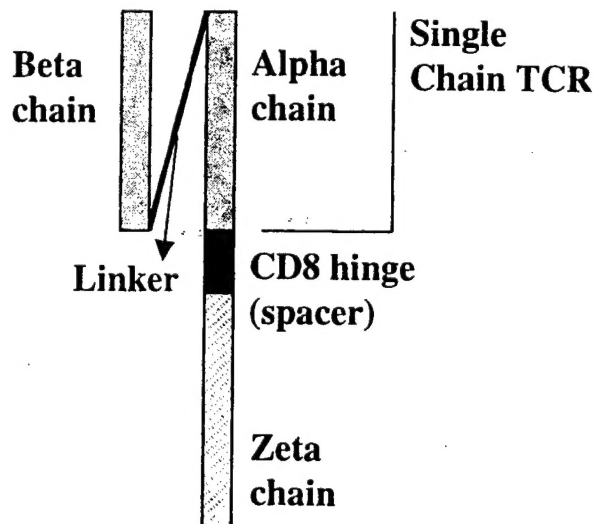


Fig 1 (A) Sequence of the single chain T cell receptor (sc-TCR).
(B) Schematic representation of the sc-TCR